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Developmental Alterations in Mast Cell Numbers and Distributions within the Thalamus of the Albino Rat

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Abstract. A total of 216 male and female albino Wistar rats *(Rattus norvegicus)* between 10 and 60 days old from 42 litters were studied to determine mast cell characteristics within the thalamus. Whereas the mean numbers of mast cells within both the parenchyma and leptomeninges (combined) did not change appreciably after 15 days of age, the appearance of mast cells within the parenchyma (and disappearance from the leptomeninges) is clearly age-dependent. Between 15 and 25 days of age, the relative numbers of mast cells within the thalamic parenchyma changed from 20 to 90% of the total mast cells within thalamic space.

Mast cells (MCs) within the brains of young albino rats [5] demonstrate a clear concentration within diencephalic space [7–9]. In 15- to 20-day-old rat brains, the majority of MCs are found within the leptomeninges of the diencephalon or within thalamic nuclei. Few, if any, MCs are apparent within hypothalamic nuclei or within any of the telencephalic (cortical or subcortical) or mesencephalic structures studied [8–10].

However, the distribution of MCs within the diencephalic leptomeninges and parenchyma undergoes clear developmental alterations. Whereas the leptomeninges contain most of the total MCs in 15-day-old brains, the parenchyma contains the predominant MC distribution in 20-day-old brains. This sudden shift of MCs towards thalamic parenchyma is not associated necessarily with statistically significant alterations in the total numbers of MCs.

As the numbers of MCs shift from the leptomeninges to the parenchyma, changes in cytoarchitecture are apparent. Between postnatal days 15 and 40, parenchymal brain MCs appear to change from small spherical entities packed with dense metachromatic materials (such as those within the leptomeninges) to larger more elliptical forms with more discernible granule distribution; usually their major axes appear along blood vessels.

The suggestion that MC numbers could account for all of the histamine in the young rat brain has been discussed by *Dropp* [1] and sup-

ported by experimentation [2, 11]. Although the variable nature of brain histamine is well known, recent data clearly indicate that different extraction procedures – that would presumably tap different sources of histamine stores – can vary concentration estimates by a factor of 10 [6]. If this pool of histamine is bound within MCs, then the morphology (or some functional measure of the tendency for degranulation) and the distribution of MCs within brain space, may be important information to explain this variability.

The precise determination of the temporal shift in MC distribution from the diencephalic meninges to the thalamic parenchyma was the primary purpose of this study. In addition, analyses were completed to determine whether: (1) numbers of MCs systematically reflected age, and (2) total MCs or relative distribution of MCs within the thalamus were influenced by litter differences, sex or various experimental manipulations.

Materials and Methods

For this study, 216 male and female albino Wistar rats *(Rattus norvegicus)* from 42 litters were killed by decapitation at 10, 15, 20, 21, 22, 25, 30, 40, or 60 days of age. All pups were selected from controlled, experimental paradigms, conducted in this laboratory over a 5-year period. Only the first litters from 80- to 120-day-old (primiparous) mothers were used; each litter was reduced to 8 pups within 24 h of birth. Mothers and pups were housed in $41 \times 25 \times 18$ cm plastic cages containing corncob bedding until weaning at 21–22 days of age. Weaned rats were housed in pairs within standard wire cages. Ralston-Purina rat chow and water were available ad libitum. The colony conditions and more detailed animal care procedures are reported elsewhere [9–10].

Three different types of experimental operations had been involved: body marking, handling and taurine consumption. In the first study [8], involving 32 subjects, pups were either marked or not marked with a permanent dye on the rear dorsal surface, daily between 5 and 20 days of age. In the second study [10], involving 60 rats, subjects were either handled or not handled for 1 min daily between 5 and 20 days of age. For the taurine study [unpublished data], 12 21-day-old pups were given 0.8% taurine in their water supply continually and 12 littermate controls were given tap water until they were killed at either 25, 30, 40, or 60 days of age. The remaining 100 rats were from experiments that did not involve an age variable (e.g., 9) and did not involve any long-term manipulation.

All brains were removed within 2 min after decapitation and fixed in ethanol-formalin-acetic acid [9] for 2 days. After stages of dehydration (ethanol) and clearing (chloroform) [9], the cerebra were infiltrated and embedded in paraffin (Tissueprep 56.5 °C). Either 5 or 10 10-μm coronal sections equally spaced between the rostral mesencephalon and septum were stained with toluidine blue 0 (in all studies but one where thionin was used). MCs, as defined by both metachromasia and morphology, were counted by traversing the entire diencephalic region of each section at $\times 400$. This fixative-staining procedure allowed clear differentiation of MCs (purple to reddish purple) against the pale blue background of the other nuclei (even the occasional darkly stained neuron was easily distinguished). Adult rat thyroid and thymus tissues, stained in the same sequences, also showed the usual numbers of MCs.

The entire surface area of each section was traversed at $\times 400$ using an Olympus microscope (trinocular model EC). Total numbers of MCs (recorded on a hand counter) for each 10-μm section were determined in the leptomeninges, by traversing the perimeter of the diencephalon and in the diencephalic parenchyma. To insure accurate counting: (1) boundaries for adjacent circular microscopic fields were followed carefully, (2) care was taken to include MCs in the small areas forming the cusp between adjacent fields, (3) the coaxial stage knobs controlling backward-forward and lateral movements of slide holder were kept tight to prevent drift, and (4) a grid eye piece was used to facilitate MC counting within a field.

The mean numbers of MCs per 10-μm section were calculated by dividing the total numbers of MCs within the leptomeninges or the parenchyma by the appropriate number of slides. The percentage of MCs within the parenchyma for each animal was determined simply by dividing the number of MCs counted within the parenchyma by the total number of MCs within the diencephalon (leptomeninges + paren-

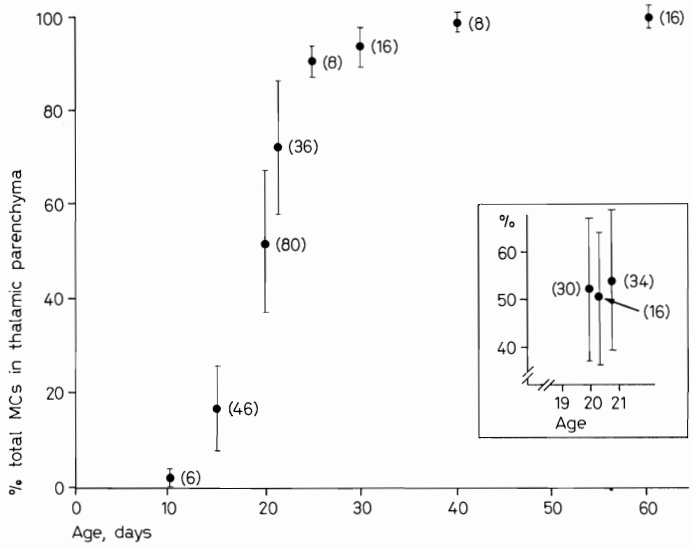

Fig. 1. Means and standard deviations (vertical bars = ± 1 SD) for the percent of total diencephalic mast cells appearing within the thalamic parenchyma as a function of age. Numbers of rats in each age group are noted in parentheses.

chyma). Data from all experiments had been recorded and stored on disk; routine program checks were used to determine any loading errors. All statistical analyses were completed by DECSYSTEM 2020 computer using SPSS software.

Results

Means and standard deviations for the percentages of total MCs found within the thalamic parenchyma of 216 rats between 10 and 60 days of age are shown in figure 1 (since none of the previous studies or the present analyses demonstrated that the experimental manipulations altered the percentage of MCs within the parenchyma with respect to the leptomeninges, all 216 rats were pooled for the construction of the figure). As can be seen, the majority of MCs within the diencephalic space of 10- and 15-day-old brains were found (usually in a single row) within the leptomeninges. By postnatal day 20, about 50% of all MCs were found within the parenchyma.

Between postnatal days 20–21 and 25, a maximum increase in the rate of MC appearance within the thalamic parenchyma has been achieved. After 30 days of age, very few MCs are found within the leptomeninges; in fact, almost all MCs can be found within the parenchyma. To demonstrate the typical reliability of the values for each age, the 80 20-day-old animals were divided into the three original experimental series (fig. 1, inset).

The mean number of MCs per 10-μm section within the diencephalon does not appear to change significantly as a function of age after postnatal day 15. As can be seen in table I, the mean numbers of MCs per 10-μm section, as determined from either 5 or 10 sections evenly spaced between the caudal and rostral diencephalic boundaries, range from 32 ± 18 to 59 ± 28. However, it must be emphasized that these values have not been corrected for the varying thalamic volume as a function of age and consequently may not reflect absolute thalamic MC estimates.

Table II displays the omega-squared estimates [3] or the r^2 values calculated by the ANOVA (3-way) program in SPSS for the amount of variability 'explained' by either age,

Table I. Means ($\overline{X}$) and standard deviations (SD) for the mean numbers of mast cells per 10-μm section in the diencephalon (leptomeninges + parenchyma) as a function of age and different experiments (n = number of subjects)

Age days	n	$\overline{X}$ MCs/10 μm	SD
10	6	100	18
15	30	57	28
15	16	59	28
20	30	40	22
20–21	34	32	18
20	16	40	17
22	36	57	44
25	8	54	27
30	16	46	22
40	8	53	11
60	16	37	12
	216		

Table II. Omega-squared estimates of variability in mean mast cell numbers and in percent mast cells in diencephalic parenchyma accounted for by litter differences, age and experimental manipulations

Experimental series	ω^2 estimates[a]	
	% total MCs	% parenchymal MCs
1976–1977 (n = 32)		
Litters (n = 8; 4/litter)	< 5	< 5
Age (15, 20 days)	13	*70*
Manipulation (daily marking vs. non-marking)	10	< 5
1977–1978 (n = 60)		
Litters (n = 15; 4/litter)	22	< 5
Age (15, 20 days)	10	*65*
Manipulation (handling vs. non-handling)	10	< 5
1978–1979 (n = 24)		
Litters (n = 6; 4/litter)	< 5	5
Age (25, 30, 40, 60 days)	5	*63*
Manipulation (0.9% taurine vs. H_2O consumption and single vs. double caging)	7	8

[a] ω^2 of $\geq 10\%$ were associated with F values that displayed $p \leq 0.01$.

litter or treatment for the total number of MCs and the relative number of MCs within thalamic parenchyma (fig. 1). Clearly, in the three experiments in which age differences were present (which involved 116 of the 216 rats), this variable accounted for more than 65% of the variability in the percentages of total MCs in the thalamic parenchyma. Age accounted for about 10% of the variability in MC numbers.

Litter differences accounted for less than 10% of the total variability in parenchymal MCs; only in one experiment did litter differences explain more than 10% of the variability in mean MC numbers. However, experimental manipulations (no handling vs. daily handling between postnatal days 5 and 20) consistently explained small but significant amounts of variability in MC numbers but not in percentages of MCs in thalamic parenchyma.

As mentioned previously [9, 10], MC numbers or percentages in the parenchyma were not correlated with body weight at a particular age (correlations with body weights across ages confound the effects from maturation). No sex differences were evident in any of the studies. Within the diencephalon, MCs were always either along the leptomeninges, as a clear discrete line of cells, or within the dorsal mid-lateral portions of the thalamus, particularly along blood vessels. Thalamic nuclei [after 4] that displayed exceptional clusters of MCs were: nucleus anterior ventralis, nucleus anterior medialis, nucleus posterior, and the nucleus ventralis (dorsal medialis).

Discussion

The redistribution of MCs within diencephalic space is clearly age-dependent. Both mathematical estimates of explained variability and visual inspection indicate that the appearance of MCs within thalamic parenchyma (and their disappearances from the leptomeninges) is age-dependent. The pattern depicted in figure 1 approximates a logistic function with the maximum acceleration (in slope) between 20 and 25 days of age.

The most obvious variable that might 'control' the appearance of MCs within the thalamic parenchyma is the growth in vascular density. *Dropp* [personal commun.] suggested this possibility on the basis of anecdotal sampling from several different species. Crude overlay of Craige's old density curves for capillarization networks [12] with the present data suggest a coincidence between the wave of MC appearances and the later stages of capillarization.

Although it is not clear which type and size of vasculature is most correlated with MC accumulation in the thalamic parenchyma after 10 days of age, the perivascular nature of MCs in 15- to 25-day-old rat brains is evident from even gross inspection. Such a conspicuous accumulation of cells that could contain brain histamine and other biopotent substances suggests neurological, humoral, and possibly immunological consequences [7] if either explosive or graded degranulation occurred.

If the MCs that appear in the parenchyma are the same cells as those that disappear from the leptomeninges, then one must consider the possibility of cell fragility during this movement. Since the greatest rate of movement appears between 15 and 25 days of age, the occurrence of degranulating substances or conditions within the proximity of the MCs could alter brain-vascular interaction.

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